FORMULATION AND EVALUATION OF GAULTHERIA OIL MICRO EMULSIONS FOR TOPICAL DELIVERY OF NSAID

SYNOPSIS

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by

Mrs. VANI S

(UIN: 2105002036)

Under the guidance of

Dr. Venkatesan N,

Professor
School of Pharmacy, Puducherry

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ABSTRACT

Introduction: Arthritis is the most prevailing pain affecting millions of people globally. It develops when the protective cartilage insulating the bones wear out over time. It is usually associated with pain and stiffness. Global statistics estimate that 1 in 5 adults suffers from arthritis. Piroxicam, a Non-Steroidal Anti-Inflammatory Drug (NSAID) is well-established drug for treating arthritis.

Aims & Objectives: In the present research, the plant oil of *Gaultheria procumbens* (family: Ericaceae), an extensively utilized ethnic drug to treat arthritis and get rid of pain, was selected to formulate microemulsions (MEs) in combination with Piroxicam. Thus the present research aimed to formulate a novel microemulsion-based hydrogel (MEH) of gaultheria oil for the topical delivery of piroxicam, to enhance solubility, dermal permeability & prolonged release, using novel gaultheria oil, thereby exhibiting increased anti-inflammatory and anti-arthritic activity. The objective of this research was to examine the suitability of gaultheria oil in formulating MEs containing non-steroidal anti-inflammatory drug (NSAID) for topical use.

Methods: The selection of excipients such as oil, surfactant, and cosurfactant was made based on the solubility studies and pseudo-ternary phase diagram. The formulation and optimization of microemulsion based hydrogel (MEH) was performed by design of experiment (DOE) using D-optimal design expert software and evaluated for qualitative and quantitative & stability studies. The optimized microemulsion (ME) was then formulated into hydrogels using hydroxy propyl methyl cellulose (HPMC) K100M in different proportions and evaluated for pH, viscosity, spreadability, drug content, diffusion study, stability studies, skin irritancy, *in-vitro* drug release, *in-vivo* anti-inflammatory, and *in-vivo* anti-arthritic activity

Results: The pre-formulation studies showed high solubility of drug (piroxicam) with gaultheria oil, tween 20 and PEG 600, thereby confirming its suitability for formulation. Fourier transform infrared spectroscopy **(**FTIR) proved drug excipient compatibility by retaining the characteristic peaks even after formulation. D-optimal design expert software was proved to be beneficial in the formulation and optimization process and matched the experimental results. Optimized piroxicam microemulsion (PM) formulation PM 8 showed satisfactory results regarding % transmittance (98.84±1.04), pH (7.41±0.01), RI

(1.4421±0.002), viscosity (469.53±0.61), % drug content (96.32±0.19), particle size (185.5 nm±0.24 nm), zeta potential (-25.34±0.51), *in-vitro* cumulative drug release in 6 h (42.62 %±0.11 %) and 12 h (86.42 %±0.21 %). The drug release kinetics study showed a zero-order release mechanism confirmed by Korsmeyer-peppas model. Stability studies as per International Council for Harmonization (ICH) guidelines demonstrated the stability of PM 8 for its physical appearance, phase separation, % transmittance, pH and % drug content for 90 days. The optimized MEH showed a pH of 7.42, viscosity 6847cps, spreadability 4.54g.cm/sec, drug content 95.81%, *in-vitro* diffusion 93.25%, *in-vitro* cumulative drug release (CDR) 42.21% in 6 hours and 89.95% in 12 hours. Finally, *in-vivo* Anti-inflammatory and anti-arthritic activity of the optimized MEH were comparable and found to be more effective than the available marketed gel.

Conclusion: Thus, it can be concluded that the present investigation study can be beneficial for improved loading of model NSAID piroxicam in the interfacial film of microemulsion with gaultheria oil as a promising vehicle aimed at the topical conveyance of drugs in the management of arthritis. Thus, MEH of piroxicam was successfully developed using Gaultheria oil as a novel encapsulating vehicle for prolonged release of the drug in treating arthritis.

Keywords: Microemulsion, gaultheria oil, arthritis, pseudo-ternary phase diagram, design expert software.

INTRODUCTION

Arthritis generally refers to joint inflammation, often accompanied by pain and stiffness. There are several types of arthritis; the predominant types include Osteoarthritis (OA) and Rheumatoid Arthritis (RA). OA is a degenerative disease that usually occurs with age, and the most affected parts are the knees, fingers, and hips. RA occurs when the body's own defense process doesn't act appropriately. It usually occurs with joints and bones, often feet and hands, leading to bone degeneration and pain It may also affect other organs such as eyes, heart, skin, or any internal organs and systems. Several other types of arthritis include gout, ankylosing spondylitis, juvenile arthritis, psoriatic arthritis & reactive arthritis. Most common symptoms of all these include redness, heat, swelling, and pain in the joints[1].

The statistics on OA prevalence during 2019- 2024 estimated about 1 in 5 adults (21.2%), and about 53.2 million people were diagnosed with OA worldwide. This chronic ailment (OA) is reported mostly among the elderly (39%), although it can affect people of any age, including children (20.3%), women (22.9%), men (16.3%), veterans (24.2%), and non-veterans (18.5%). OA is expected to increase due to an aging population and increased obesity[2].

To control the symptoms of arthritis, drug therapy must be initiated to maintain a constant effect. This is achieved by using Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), by administering repeated doses throughout the day. This leads to accumulation of the drug to 'peak' plasma concentrations, often above the recommended maximum dose, leading to safety concerns[3]. Piroxicam, is a BCS (Biopharmaceutics Classification System) class II drug, is a widely used Non-Steroidal Anti-Inflammatory Drug (NSAID). Chemically it is an oxicam derivative, which is a well-established drug in the treatment of arthritis. Piroxicam is proven to be superior to other classes of NSAIDs such as ibuprofen, naproxen, indomethacin, aspirin, ketoprofen, diclofenac, phenyl butazone, and sulindac[4]. Studies have demonstrated its anti-inflammatory & anti-arthritic activity in oral and topical formulations[5]. However, with oral administration of piroxicam, gastrointestinal problems are the most common side effects, and also undergo significant degradation, resulting in less efficacy of the drug formulation[6]. These main disadvantages intensified the search for an alternative topical drug delivery system. Thus, piroxicam was selected for the study, which has low solubility and high lipophilicity, making it a suitable candidate for topical administration.

Gandhapura" (gaultheria oil) or simply Indian winter green, comprises methyl salicylate (organic ester), which is a topical analgesic parallel to aspirin in treating sprains, aches, and

pain[7]. It is used in Chinese folk medicine to prepare many pharmaceutical creams for topical use for extreme and mild pain[8]. Realizing the economic and pharmaceutical value of gaultheria oil, it must be encouraged to be used in pharmaceutical formulations. So, creams, ointments, or gels containing this oil can be a good relief for the pain[9]. Hence, gaultheria oil was selected for the encapsulation of piroxicam, with a view to the combined effect of gaultheria oil and piroxicam may result in better anti-inflammatory and anti-arthritic activity, which could be beneficial in treating arthritis.

The skin acts as a natural barrier for the topical delivery of the drugs[10]. Taking it into consideration, microemulsions were formulated to enhance drug penetration through the skin by dissolving lipids in the skin's barrier layer, namely the stratum corneum[11]. These microemulsions are thermodynamically stable, clear dispersions of oil and water, stabilized by surfactant and co-surfactant with particle sizes ranging from 100 nm - 200 nm. Due to which they pose low interfacial tension, high diffusion, and higher absorption rates[12]. But the main disadvantage of microemulsions is their stability problem, due to low viscosity[13]. Semisolids (creams, ointments, and lotions) are frequently used as topical medications that have several drawbacks, such as causing discomfort to the patient when applied, being sticky and needing rubbing, and having a lack of stability[14]. As a result of all these drawbacks of semisolids, the usage of hydrogels has demand in newer medicine and cosmetics[15]. Despite the several advantages of hydrogels, the administration of lipophilic moieties is limited[16]. As a result, a microemulsion-based hydrogel concept was used to overcome this limitation, wherein a hydrophobic therapeutic moiety can be effectively fabricated into hydrogels[17]. Using the drug/oil/water or drug/water/oil emulsions technique, hydrophobic drug moieties can be fabricated into microemulsion-based hydrogels (MEH) using natural polymers such as HPMC K 100 M[18].

The usual dose of piroxicam is 20 mg/day for 2- 3 weeks to treat OA[19]. Presently available topical formulations of piroxicam in the market include gel (0.5%), showing very slow absorption following topical administration, and also require repeated administration[20,21]. Hence, to increase the topical skin permeability, prolonged release, and enhanced anti-inflammatory and anti-arthritic activity, microemulsion-based hydrogels of gaultheria oil-piroxicam were planned to be formulated in the present research work.

AIM:

The present research aims to formulate microemulsions of gaultheria oil in combination with piroxicam to develop microemulsion-based hydrogels (MEH) of piroxicam & gaultheria oil for topical application with a view to increased anti-inflammatory & anti-arthritic activity.
Novel formulation development by combining plant oil with NSAID that confer maximum benefit in treating arthritis.

OBJECTIVES:

To check the suitability of gaultheria oil in formulation development.
To confirm the suitability of D-optimal design expert software in the formulation and optimization of microemulsions.
To achieve more entrapment efficiency, drug loading capacity, and prolonged release of microemulsions.
To incorporate microemulsions into a hydrogel matrix for developing novel microemulsion-based hydrogels for enhanced anti-arthritic activity.
To find a novel encapsulating vehicle in the formulation of microemulsion-based hydrogels.
To develop a stable, novel formulation for treating arthritis.

HYPOTHESIS & RESEARCH QUESTIONS

"Gandhapura/ Tailapatra" (Gaultheria oil) or simply Indian winter green tree (obtained from plants of *gaultheria procumbens* & *gaultheria fragrantissima*, belonging to the family Euphorbiacea), which is native to Himalaya mainly Central and Eastern, Nilgiris and north eastern Khashi hills. It is an Evergreen plant found even at the height of 5000 to 6000 meters and beers flower with white purplish color. The leaves of Wintergreen contain aromatic essential oils. It is aromatic primarily due to the presence of methyl salicylate, which is extracted through fermentation and steam distillation process of the material. This plant comprises of methyl salicylate (organic ester), which is a topical analgesic parallel to aspirin in treating sprains, aches, and pain. It is used in Chinese folk to prepare many pharmaceutical creams for topical use for extreme and mild pain.

Hence in this research, an attempt was made to formulate a novel topical formulation by using gaultheria oil as a vehicle for the entrapment of piroxicam, with an objective of enhanced anti-inflammatory and anti-arthritic activities. Realizing the economic and pharmaceutical value of gaultheria oil, it must be encouraged to be used in pharmaceutical formulations in India as well.

Since the genus is important and has tremendous commercial potential, hence immediate attention should be paid for its conservation and sustainable utilization. Methyl salicylate which is the chief component of Wintergreen Oil is an effective analgesic and helps minimalizing inflammatory reactions in the body leading to pain. So creams, ointments or gels containing the oil can be a good reliever for the same. It fulfills its pharmaceutical purposes as its anti-inflammatory abilities also work well against arthritis.

Piroxicam, a non-steroidal Anti-Inflammatory Drug (NSAID) is a BCS (Biopharmaceutics Classification System) class II drug, most preferred drug in the treatment of arthritis & proven to be superior when compared to other NSAIDs. Presently available topical formulations of piroxicam in the market include gel (0.5%), showing very slow absorption & require repeated administration.

Thus, an attempt was made in this research to combine piroxicam and Gaultheria oil that could result in improved anti-inflammatory and anti-arthritic activity, which could be beneficial in treating arthritis.

REVIEW OF LITERATURE

Arthritis prevalence was highest among adults reporting no leisure time physical activity (30.9%) compared to those who are insufficiently active (27.0%) or who meet physical activity recommendations (18.8%). Arthritis symptoms can be a barrier to physical activity, and inactivity among people with arthritis is associated with conditions such as cardiovascular disease, diabetes, obesity, and functional limitations. However, regular physical activity can be an important way to reduce pain, improve function, and to manage symptoms for people with arthritis and other chronic conditions new formulation developments required[22].

GC–MS analysis of gaultheria oil showed that methyl salicylate (96.90%) was the main component of the oil. Essential oil inhibited the growth of all microorganisms tested, i.e. food and oral bacteria and fungi, respectively (MIC 0.18–3.00 mg/ml and MBC 1.25–4.00 mg/ml; MIC 0.73–5.00 mg/ml and MFC 2.92–26.67 mg/ml); The oil effectively inhibited the biofilm formation of oral *Streptococcus mutans* and *Candida albicans* as well (MIC 25.00 MBC 50.00 mg/ml; MIC 12.50, MFC 50.00 mg/ml). In addition, oil exhibited a dose-dependent DPPH-radical-scavenging activity with IC_{50} value of 30.61 mg/ml. The specific fluorescence probe *2-[6-(4'-amino)phenoxy-3H-xanten-3-on-9-yl] benzoic acid* (APF) and the spin trap *5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide* (DEPMPO), capable of simultaneous detection of different free radical specie, were used in antiradical activity of the oil measurements. Oil showed a moderate antiradical activity, reducing the quantity of produced hydroxyl radicals to about 20% of the initial value. This study succeeds in creating directly comparable and quantitative data for the oil insufficiently examined so far[23].

The o/w microemulsion formulations for the dermal delivery of TNX were developed and characterized. Ex vivo permeation rate of both microemulsion formulations was significantly higher when compared to conventional topical formulations TNX 01 and TNX 02 ($p<0.001$). In addition to better permeation, microemulsion formulations elicited significant ($p<0.001$) anti-arthritic and anti-inflammatory efficacy in various animal models of arthritis and inflammation, and therapeutic response was comparable to oral formulation of drug. Moreover, histopathology studies revealed dermal safety of both microemulsion formulations. In conclusion, the study demonstrated that microemulsion formulations of TNX could be a potential alternative to oral formulation of drug[24].

Piroxicam Emulgel formulations prepared with three different gelling agents: Carbopol 934, HPMCK4M and Poloxamer 407 showed acceptable physical properties and drug release study. All prepared Emulgel showed acceptable physical properties concerning colour, homogeneity, consistency, spreadability and pH value. Among all gel formulations, HPMCK4M Emulgels shows superior drug release after that Poloxamer 407 shows decreasing order of drug release. In all this Emulgel formulation, the drug release was decreased with increase in gelling agent concentration because polymer concentration increases, viscosity increases. Viscosity is negatively related to the release of active substance (Piroxicam) from formulations. Stability studies in all gel formulations showed that, the physical appearance, drug content, pH, rheological properties, and drug release in all gel formulations remain unchanged upon storage for two months[25].

METHODOLOGY

Materials

Piroxicam working reference standard was obtained as a gift sample from Alcon Biosciences Private Limited, Maharashtra, India. Gaultheria oil was procured from Tamil Nadu Tea Plantation Corporation Ltd., Ooty, Tamil Nadu, India. Tween 20 purchased from Industrial chemicals, Hyderabad, India. PEG 600 purchased from Matangi Industries, GIDC, Vatva, Ahmadabad, Gujarat, India. HPMC K100M was procured from Bangalore fine chemicals, Bangalore, Karnataka, India. The solvents and chemicals employed remained analytical grade. Water employed in the research was High Performance Liquid Chromatography (HPLC) grade. All other chemicals employed were of analytical grade. Instruments employed were UV 1900 Shimadzu corporation Japan, Bruker ATR Germany for FTIR, Shimadzu AUX-224 electronic analytical balance, Consolidated electrical Industries pH meter, Electrolab–Trust E-08 Servewell instrument for dissolution testing, Remi magnetic stirrer, Anton Scientific Franz diffusion cell, Videocon refrigerator, Equitron/7047 DT-043 Ecogain series hot air oven.

Methods

Solubility study of Piroxicam

Screening of excipients such as oils, surfactants and co-surfactants, were performed for Piroxicam solubility. It was performed by taking more amount of Piroxicam (about 200 mg) in microtube comprising 2 ml of each excipient and screw capped, stirred at 100 rotations per minute (rpm) for 72 hours (h), maintained at 37° ± 0.5° C. Each of these vials were centrifuged at 3000 rpm for 30 minutes (min). Allowed to stand for 30 min, transfer the clear supernatant liquid and measure its absorbance by using Ultra violet-Visible (UV) spectrophotometer at 354 nm[26].

Creation of pseudo-ternary phase diagrams

The equilibrium region of microemulsion (ME) were constructed using water titration method. The selected oil (gaultheria oil), surfactant (Tween 20) and co-surfactant (PEG 600) mix (S-mix with 1:1, 1:2 and 2:1 ratios). Each S-mix ratio was mixed with oil in the ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 and titrated with distilled water. Once a clear, transparent mixture was obtained, it was measured as monophasic system and considered as

points of equilibrium for constructing phase diagrams. The formulation with highest equilibrium area was considered as an optimized proportion for formulation of MEs[27].

Formulation, optimization and evaluation of Microemulsions (MEs)

MEs were prepared by mixing the Gaultheria oil and water with S-mix (Tween 20 : PEG 600 in 1:1 ratio) by constant stirring on a magnetic stirrer set at 300 rpm, 37° C ± 0.5° C for 30 minutes, followed by slow addition of pre-determined quantity of drug[28]. Then the optimization of MEs was executed by Design of Experiments (DOE), D-optimal 13.0.5.0 design expert software, whereby excipients, such as oil (Gaultheria oil), surfactant (Tween 20) and co-surfactant (PEG 600) optimal concentrations were obtained from a stable ME. Then these ME's were evaluated for Qualitative, Quantitative and stability studies. Based upon the results optimized batch has been taken for formulation into hydrogels for further development.

Formulation of Microemulsion Based Hydrogels (MEH)

HPMC K100 M used as a hydrogel matrix to construct MEH for improving the viscosity of microemulsion for topical administration, while also enabling drug delivery and enhanced penetration. Hydrogel was first soaked in water for 4 hours to swell. To this, optimized ME was slowly added with continuous stirring until a clear viscous solution is obtained. Finally, MEH were obtained[29]. Confirmation of the hydrogel was obtained once homogeneous mixture that did not vary in the meniscus when tilted to an angle of 90°. Hydrogel (HPMC K100) concentrations of 1%, 1.5% and 2.0% were used as shown in table 1.

Table 1: Composition of MEH

COMPONENTS	MEH-A	MEH-B	MEH-C
	% w/w	**% w/w**	**% w/w**
Piroxicam	0.5	0.5	0.5
Gaultheria oil	48.4	48.46	48.46
Tween 20+ PEG 600 (S-mix) (1:1)	26.10	26.10	26.10
Water	25.38	25.38	25.38
HPMC K100 M	1.0	1.5	2.0

Evaluation of MEH

Physical appearance

The formulated MEH were evaluated for colour, consistency, and homogenecity and phase separation. A simple visual examination was performed to check for the presence of any clumps or regions of different densities[30]. They should be consistent with uniform texture and should not be any phase separation.

pH

The pH was determined using calibrated digital pH meter (3-point calibration). A known quantity of MEH was dispersed in 25 ml water and pH was determined after 10 minutes of contact with the electrode. Ideal value for hydrogel meant for topical application ranges between 6 and 8[31]. The measurement was taken in triplicate and mean value was reported.

Viscosity

Brookfield viscometer was used to measure the viscosity of MEH using T-bar spindle (T-95). For viscosity measurement, the sample is taken in sample holder provided with the instrument, set at 100 rpm and 25° C ± 0.5° C for 60 seconds[32]. The measurement was taken in triplicate and mean value was reported.

Spreadability

Spreadability was performed in order to access the ease of application on to the skin. It's a crucial factor in dermal products that determines the hydrogels effectiveness and user experience. This study was performed by "slip and drag method" using two glass slides. A ground glass is used and hydrogel is place on it. Another glass slide is placed on top of it. 100 g weight was placed on the upper glass slide for 5 minutes to compress the gel into uniform thickness. The time in seconds required to separate the two slides gives the measure of spreadability.

It is calculated by using the formula: $S = (m \times L) / t$

Where, S = spreadability, m = weight applied on glass slide, L = length of the glass slide and t = time in seconds[33].

Drug content

MEH equivalent to 20 mg drug was taken in 100 ml volumetric flask. Dissolve with 20 ml methanol and sonicated for 15 minutes. Make up the volume with methanol. It is then filtered through Whatman filter paper 41. Further, it is suitably diluted with methanol so as to get a concentration 10 µg/ ml. Measure the absorbance at 334 nm[34]. The measurement was taken in triplicate and mean value was reported.

***In-vitro* diffusion studies**

It is performed to study the drug release from the dosage form. *In-vitro* drug diffusion study was performed by using Franz diffusion cell (15 ml cell volume) with cellophane membrane (≃ 25 cm^2). Cellophane membrane was first washed in running water, and then soaked in distilled water for 24 hours before used for diffusion study. Apply 200 mg MEH formulation on to the donor compartment. The receptor compartment is filled with phosphate buffer pH 7.4. Then stirred on a magnetic stirrer at 300 rpm and 37° C ± 0.5° C[35]. Aliquots 1.0 ml was taken from receptor compartment at suitable time intervals of 0 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 8 hours and UV absorbance was measured at 354 nm.

***In-vitro* cumulative drug release studies**

It is performed to study the prolonged release pattern of drug from the dosage form. Performed by using dissolution apparatus with paddle over disc assembly, with 900 ml of phosphate buffer pH 7.4 as dissolution media set at 100 rpm and 37° C ± 0.5° C[36]. Aliquots 10 ml was withdrawn at suitable time intervals of 30 minutes, 1 hour, 4 hours, 8 hours and 12 hours and UV absorbance was measured at 354 nm. *In-vitro* cumulative drug release was found at the end of 12 hours.

Stability studies

Accelerated stability studies were performed as per International Conference on Harmonization (ICH) guidelines. The MEH formulations were placed in refrigerator (5° ± 2° C), at room temperature (30° ± 2° C and relative humidity 65 ± 5% RH) and in climatic chamber (40° ± 2° C and relative humidity 75 ± 5% RH) for 90 days[37]. After 90 days, the samples were evaluated for pH and drug content.

Skin irritancy test

As the formulation was intended for dermal application, skin irritancy should be tested. Skin irritation tests were conducted in rabbits to determine irritancy after single application of microemulsion-based hydrogel. The back of rabbits after depilation (approximately 10 x 15 cm area) was used in this experiment. About 0.5 g of commercial piroxicam cream and optimized microemulsion-based hydrogel was applied on two different rabbits and then the applied area was covered with gauze and adhesive bandage. The formulation was removed after 24 hours and the exposed skin was graded for formation of edema and erythema. Scoring was repeated 72 hours later. Based on the scoring, the formulation was graded as 'non-irritant', 'slightly irritant', 'irritant' and 'highly irritant' & expressed as 0, 1, 2 & 3 as irritation index respectively as shown below[38].

Table 2: Scores of erythema and edema in skin irritancy test

Score	**Erythema**	**Edema**	**Inference**
0	No erythema	No edema	Non-irritant
1	Slightly erythema	Slight edema	Slightly irritant
2	Moderate erythema	Moderate edema	Irritant
3	Severe erythema	Severe edema	Highly irritant

***In-vivo* anti-inflammatory activity**

Croton oil induced ear edema model was well established method to study *in-vivo* anti-inflammatory activity. Croton oil (*Crotonis oleum*) is oil obtained from the seeds of *Croton tiglium*, a tree belonging to Euphorbiaceae family. Due to its irritating properties, it is widely used on laboratory animals to induce ear edema and to study pain and anti-inflammatory activity. In this test, Swiss albino mice weighing about 25 to 30 grams were employed. The animals were housed separately at 22 °C ± 2 °C on 12-hours light/dark cycle with free access to rodent, diet and water. Mice were divided into 4 groups with 6 mice in each as shown in table below.

Table 3: Anti-inflammatory activity of MEH (by Croton oil induced ear edema model in mice)

Group Name	Treatment Group	Number of animals in each group
I	Edema control group	6 mice
II	Standard drug group	6 mice
III	Optimized formulation group	6 mice
IV	Marketed formulation group	6 mice
	TOTAL	**24 mice**

Edema control group I received 15 µl of acetonic solution of croton oil which is applied on inner and outer surface of right ear of each mouse (about 1 cm^2 areas). Group II applied with Dexamethasone 0.08 mg/ear, group III applied with optimized hydrogel and group IV with marketed formulation. After 6 hours of application the ears were removed (6mm diameter) and immediately weighed. The weight difference between the treated and untreated ears was recorded and the difference gives the measure of inflammatory edema. Then the percentage inhibition was calculated by using the following formula:

$$\% \text{ inhibition} = (E_{control} - E_{treated}) / E_{control} \times 100$$

Where, $E_{control}$ and $E_{treated}$ is the measure of ear edema (ear weight in mg) of control group and treated groups respectively[39].

in-vivo anti-arthritic activity

In-vivo anti-arthritic activity was performed using Complete Freund's adjuvant (CFA) model. This model accurately depicts the features of clinical rheumatoid arthritis. This model is superior to other models for evaluating the efficacy of anti-rheumatic medicines because of the delayed systemic response to Freund's adjuvant. Drugs for treating inflammation which are given on a long-term basis could be tested using rats. CFA is water in oil emulsion consisting of heat killed mycobacterial (inactivated) suspended in a mineral oil.

In this test, Wistar rats weighing about 150 to 200 grams, aged about 5 to 8 weeks were employed. The animals were housed separately at 22° C ± 2° C on 12-hours light/dark cycle with free access to rodent, diet and water. Rats were divided into 4 groups with 6 rats in each as shown in table below.

Table 4: Anti-arthritic activity of MEH (by CFA induced paw edema model in rats)

Group Name	Treatment Group	Number of animals in each group
I	Normal control group	6 rats
II	Arthritic control group	6 rats
III	Optimized formulation group	6 rats
IV	Marketed formulation group	6 rats
	TOTAL	**24 rats**

Group I was kept as a normal control group, without receiving any drug. Group II, arthritic control group, which received 0.1 ml of CFA given subcutaneously (S/C) into the sub plantar region of the right hind paw. Group III received CFA (0.1 ml as above) and after 30 minutes, was applied with optimized hydrogel. Similarly, group IV also received CFA (0.1 ml as above) and after 30 minutes, was applied with the marketed formulation. The changes in the paw size (thickness) were measured at 0, 4, 7, and 14 days, using Vernier calipers and the results were recorded in millimeters[40,41].

Biochemical and Hematological parameters

Biochemical and Hematological study is the quantitative measurement of biochemical substances in biological samples, mainly to assess the health, disease or treatment response. On 21st day, blood is collected (without anticoagulant) from tail vein of rats under ether anaesthesia. The serum was separated by centrifugation at 10,000 rpm for 15 minutes. Samples were then analyzed for Hemoglobin (Hb), Red Blood Cells Count (RBCs), Lymphocytes, Erythrocyte Sedimentation Rate (ESR), White Blood Cells Count (WBCs), Neutrophils, Serum Glutamic Pyruvate Transaminase (SGPT), Serum Glutamic Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP) and C-reactive Protein (CRP) using specific methods[42].

Statistical analysis

The data obtained in each experiment was analyzed by one way analysis of variance (ANOVA) by means of student's t-test. The values were said to be statistically significant, when the P value ≤ 0.05. It was observed that the P value of all responses for linear model was found to be ≤ 0.05. Hence, the results were found to be significant.

RESULTS AND DISCUSSIONS

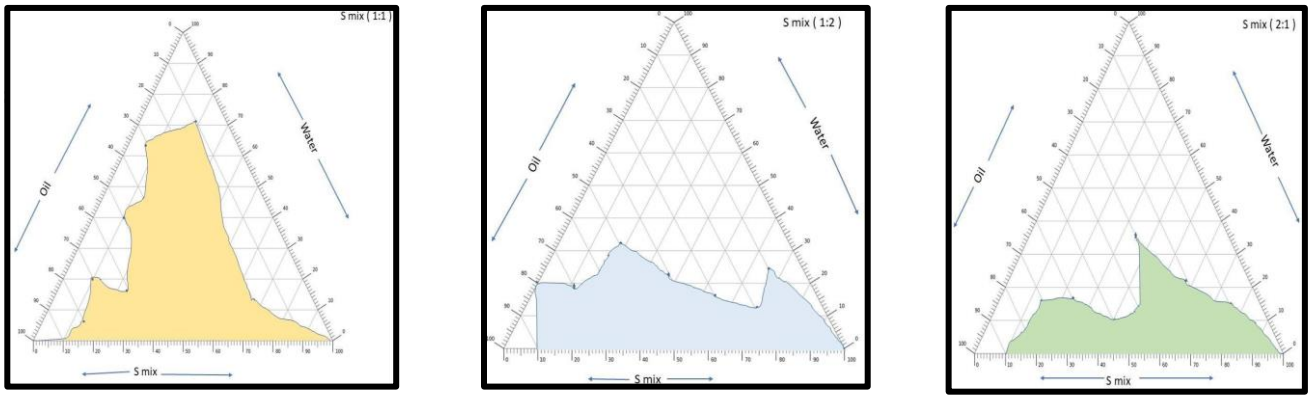
Solubility study of Piroxicam

The UV absorbance of Piroxicam was found to be more with Gaultheria oil, Tween 20 and PEG 600, when compared to other oils, surfactants and co-surfactants tested as shown below.

Table 5: UV absorbance of piroxicam in different excipients (oils, surfactant & co-surfactants)

Excipients	**UV absorbance at 354 nm (n=3)**
Oils	
Clove oil	0.6127
Almond oil	0.3383
Cypress oil	0.6245
Olive oil	0.2328
Gaultheria oil	0.8127
Surfactants	
Tween 20	0.2313
Tween 80	0.0020
Co-surfactants	
PEG 300	0.3158
Ethanol	0.2979
PEG 600	0.7513

Fig 1. Pseudo-ternary phase diagrams



Based on the highest equilibrium area obtained from pseudo-ternary phase diagrams, S-mix ratio of 1:1, was considered as optimized concentration and selected for ME formulations.

Formulation & Optimization of MEs by D-Optimal software

Table 6: DOE analysis parameters for gaultheria oil- piroxicam microemulsions (PMs)

For microemulsion formulation design	
QBD	Gaultheria oil- piroxicam microemulsions
Design	D-Optimal
Model	Quadratic
Tool	DOE 13
Version	version 13.0.5.0
Variables	%Water, %oil, %Smix
Response	Globule size, % Transmittance, *in-vitro* drug release (%) in 6hours &12hours

Table 7: Optimization protocol design by DOE for gaultheria oil- piroxicam microemulsions (PMs) with predicted responses (factors versus responses)

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
Run	A:Oil	B:Smix	C:Water	R1-Globule size	R2-Transmittance	R3 - *in-vitro* drug release in 6 hours	R4 - *in-vitro* drug release in 12 hours
	ml	ml	ml	nm	%	%	%
1	7	4	2	190.41	95.43	41.41	81.38
2	6.3	3	3	178.41	96.42	40.52	80.22
3	6	3	2.3	183.43	98.25	36.71	76.51
4	6	4	2	180.21	97.25	36.48	76.32
5	6.4	3.4	2	183.21	96.64	40.18	80.62
6	6	3	3	182.86	97.22	36.79	76.78
7	6.4	4	2.5	180.79	96.24	41.31	81.37
8	7	4	3	189.95	95.26	41.12	80.96
9	6	4	2	185.09	97.45	36.43	76.26
10	6	3.6	3	181.56	97.45	36.43	76.28
11	7	3.4	2.6	191.08	97.09	40.67	80.76
12	7	3	2	193.24	95.29	40.59	80.31

Table 8: DOE analysis of factors

Factor	Name	Units	Type	Sub Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Oil	ml	Numeric	Continuous	6.00	7.00	-1 ↔ 6.00	+1 ↔ 7.00	6.5	0.4534
B	Smix	ml	Numeric	Continuous	3.00	4.00	-1 ↔ 3.00	+1 ↔ 4.00	3.5	0.4547
C	Water	ml	Numeric	Continuous	2.00	3.00	-1 ↔ 2.00	+1 ↔ 3.00	2.5	0.4546

Table 9: DOE analysis of responses

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.
R1	R1-Globule size	nm	12.00	178.41	193.24	185.82	0.9112
R2	R2-Transmittance	%	12.00	95.26	98.25	96.75	0.9633
R3	R3 - *in-vitro* drug release in 6 hours	%	12.00	36.43	41.41	38.92	0.2284
R4	R4 - *in-vitro* drug release in 12 hours	%	12.00	76.26	81.38	78.82	0.2861

Table 10: Comparison of physicochemical properties of piroxicam WRS & IPRS

Identification test	Observed result of piroxicam WRS	Reported standard of piroxicam IPRS
Appearance	Off-white to light yellow powder	Off-white powder
Colour	White	White / off-white
Odor	Odorless	Odorless

Table 11: Melting point determination of piroxicam WRS & IPRS

Sl No	M.P Observed	M.P of Reported Standard
1	198 ºC	198 ºC-200 ºC
2	199 ºC	
3	198 ºC	
Average	198.33ºC	

Table 12: Characterization of PMs for percentage transmittance, pH, refractive index, viscosity and drug content

Formulation code	Transmittance (%)	pH	Refractive index	Viscosity (cp)	Drug content (%)
PM1	84.78±1.19	6.24 ±0.01	1.4236±0.004	896.36±0.74	88.36±0.45
PM 2	86.34±1.45	6.35 ±0.02	1.4234±0.009	810.16±0.57	92.32±0.81
PM 3	68.61±1.24	7.12 ±0.03	1.4187±0.005	789.33±0.63	86.53±0.73
PM 4	76.58±2.17	6.84 ±0.02	1.4260±0.007	604.66±0.82	94.25±0.94
PM 5	85.33±1.75	7.38±0.01	1.4310±0.009	671.57±0.74	88.28±0.62
PM 6	77.51±2.14	6.54 ±0.01	1.4187±0.008	577.12±0.31	87.42±0.41
PM 7	68.33±1.61	7.34 ±0.01	1.4206±0.006	522.71±0.55	92.19±0.82
PM 8	98.84±1.04	7.41±0.01	1.4421±0.002	469.53±0.61	96.32±0.19
PM 9	67.36 ±2.54	5.98 ±0.02	1.4311±0.014	417.29±0.98	84.54 ±0.84
PM 10	77.21 ±2.85	6.57 ±0.02	1.4295±0.021	395.74±0.74	95.53 ±0.77
PM 11	88.12 ±2.47	7.38 ±0.02	1.4272±0.016	363.52±0.94	91.33 ±0.54
PM 12	86.5 ±2.21	6.48 ± 0.01	1.4275±0.019	261.16±0.57	84.42 ±0.35

(All values are mean± SD, n=3) PM: Gaultheria oil- piroxicam microemulsions, cp: Centi poise

Table 13: *In-vitro* cumulative drug release (%) studies of PMs for 6 hours and 12 hours

Formulation code	Particle size (nm)	Zeta potential (Mv)	*In-vitro* cumulative drug release in 6 hours (%)	*In-vitro* cumulative drug release in 12 hours (%)
PM1	185.1±0.05	-25.04±1.24	41.41±0.12	81.38±0.24
PM 2	184.9±0.02	-25.17 ±2.37	40.52±0.32	80.22±0.38
PM 3	183.2 ± 0.08	-25.30 ±1.78	36.71±0.25	76.51±0.59
PM 4	180.1 ±0.06	-25.24±1.69	36.48±0.45	76.32±0.41
PM 5	181.5 ±0.11	-25.33±1.86	40.18±0.64	80.62±0.27
PM 6	182.2 ±0.18	-25.28±2.72	36.79±0.21	76.78±0.62
PM 7	185.0 ±0.21	-25.16±2.11	40.31±0.37	81.37±0.47
PM 8	185.5±0.24	-25.34±0.51	42.62±0.11	86.42±0.21
PM 9	178.9 ±0.45	-25.22±1.08	36.43±0.28	76.26±0.36
PM 10	188.7 ±0.35	-25.74±1.56	36.58±0.51	76.67±0.48
PM 11	187.5 ±0.61	-25.21±1.34	40.67±0.95	80.76±0.97
PM 12	185.6 ±0.48	-25.28±1.71	40.59±0.99	80.31±0.91

(All values are mean ±SD, n=3) PM: Gaultheria oil- piroxicam microemulsions, nm: nanometer, Mv: mili volts

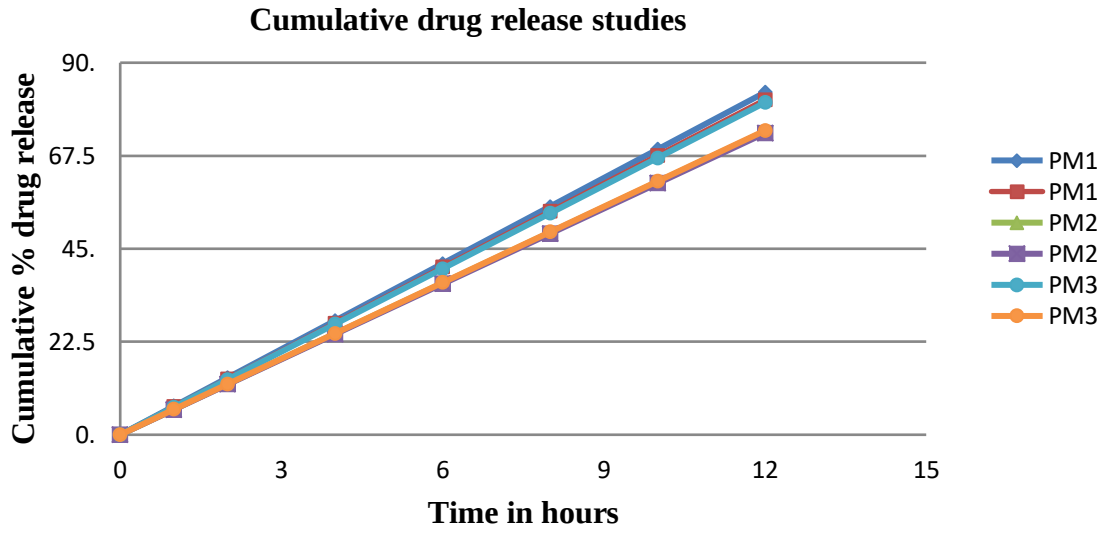


Fig 2: *In-vitro* cumulative drug release (%) studies of PMs

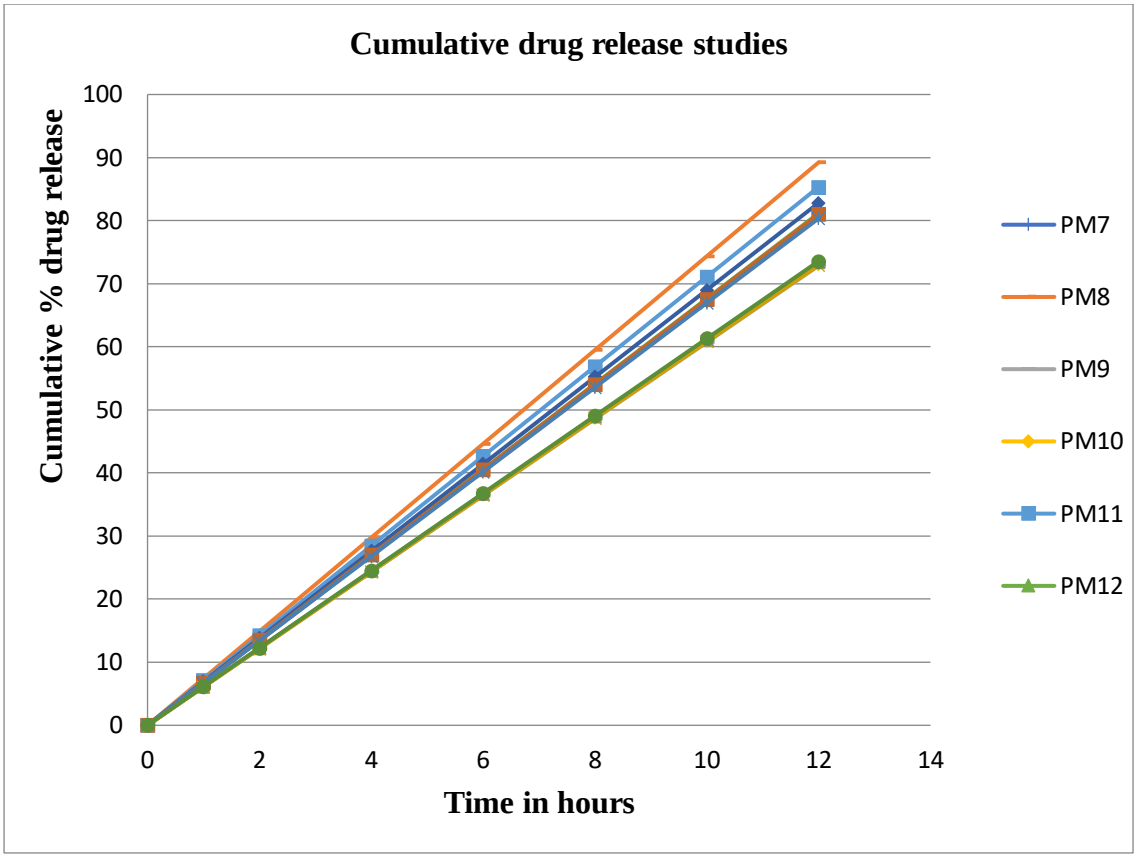


Fig 3: *In-vitro* cumulative drug release (%) studies of PMs

Table 14: Drug release kinetics of optimized PM8 formulation

Formulation code	**R^2 value**					
	Zero order	First order	Higuchi	Hixon crowell	Korsmeyer peppas	
					R^2 value	n value
PM8	0.9908	0.9102	0.9656	0.9745	0.9784	0.3064

(PM8: Gaultheria oil- piroxicam microemulsion formulation 8)

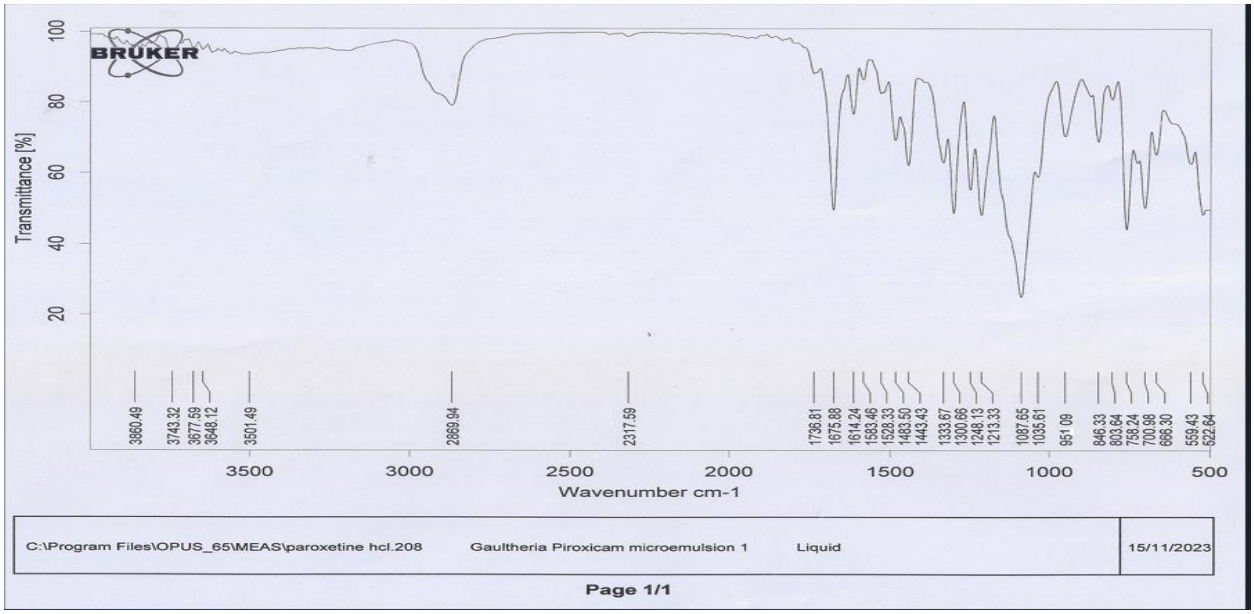


Fig 4: FTIR of optimized formulation of PM8

Table 15: Stability studies of optimized formulation PM8

Temperature	Days	Appearance	Centrifuge	Transmittance (%)	pH	Drug content (%)
5 ±2°C	30	No phase separation	Homogenous	98.81±0.02	7.43±0.01	96.27±0.11
	60	No phase separation	Homogenous	98.83±0.02	7.44±0.02	96.31±0.15
	90	No phase separation	Homogenous	98.82±0.03	7.42±0.03	96.29±0.16
30°C ±2°C and 65 ±5%RH	30	No phase separation	Homogenous	98.80±0.01	7.41±0.02	96.30±0.09
	60	No phase separation	Homogenous	98.78±0.02	7.43±0.01	96.29±0.10
	90	No phase separation	Homogenous	98.79±0.03	7.42±0.01	96.28±0.07
40°C ±2°C and 75 ±5%RH	30	No phase separation	Homogenous	98.83±0.01	7.42±0.01	96.31±0.18
	60	No phase separation	Homogenous	98.81±0.01	7.41±0.01	96.32±0.14
	90	No phase separation	Homogenous	98.80±0.02	7.43 ±0.02	96.30±0.11

(All values are mean ± SD, n=3), PM8: Gaultheria oil- piroxicam microemulsion formulation 8

Based the evaluation results for globule size, % transmittance, % cumulative drug release (CDR) for 6 hours and 12 hours.ME batch of PM8 was found to be optimized batch, comprising of Gaultheria oil 48.46%, S-mix 26.10% and water 25.38%.

Fitting of data to the Box-Behnken Design

Figure 5: 3D Surface graphs of gaultheria oil- piroxicam microemulsions globule size response verses factors oil, S-mix and water i.e. AB, BC, CA

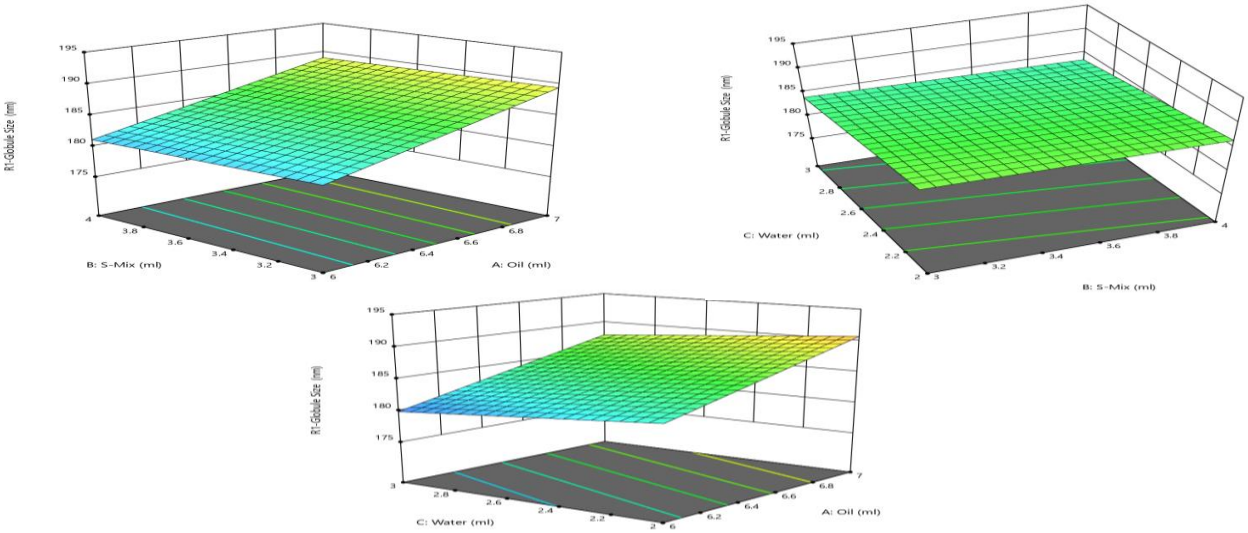


Figure 6: 3D Surface graphs of gaultheria oil- piroxicam microemulsions % transmittance response verses factors oil, S-mix and water i.e. AB, BC, CA

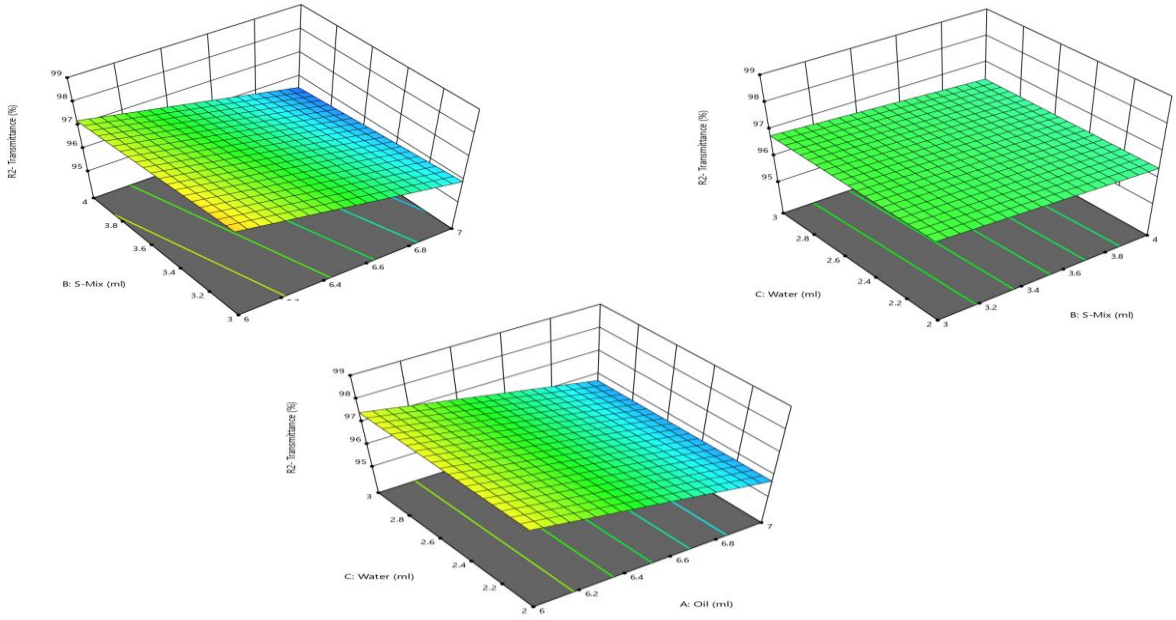


Figure 7: 3D Surface graphs of gaultheria oil- piroxicam microemulsions % CDR for 6 hours verses factors oil, S-mix and water i.e. AB, BC, CA

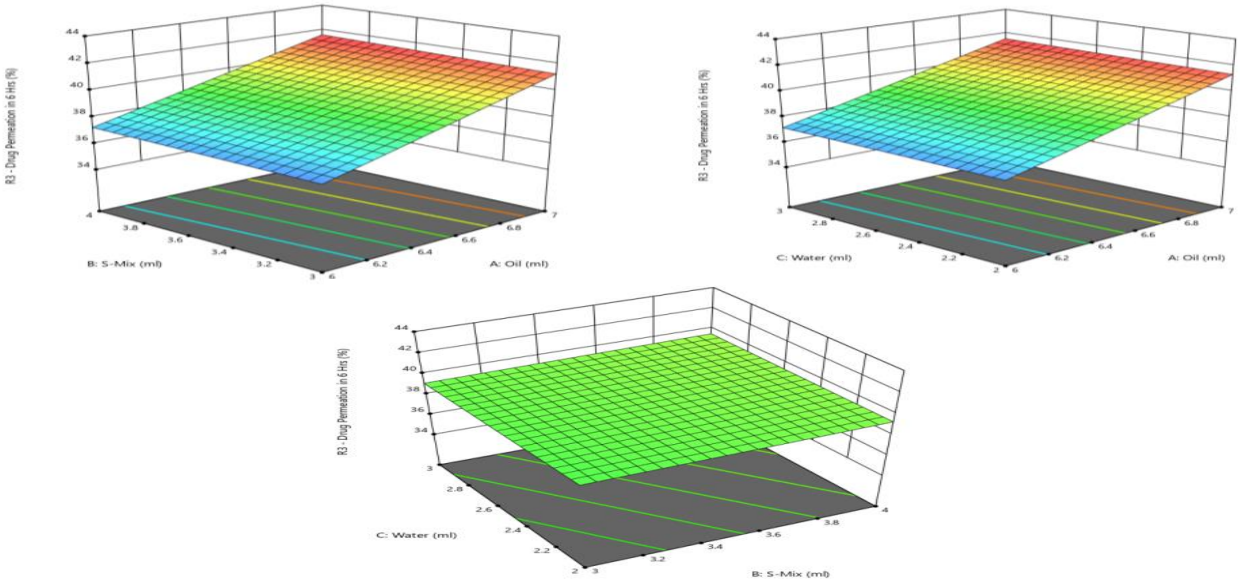
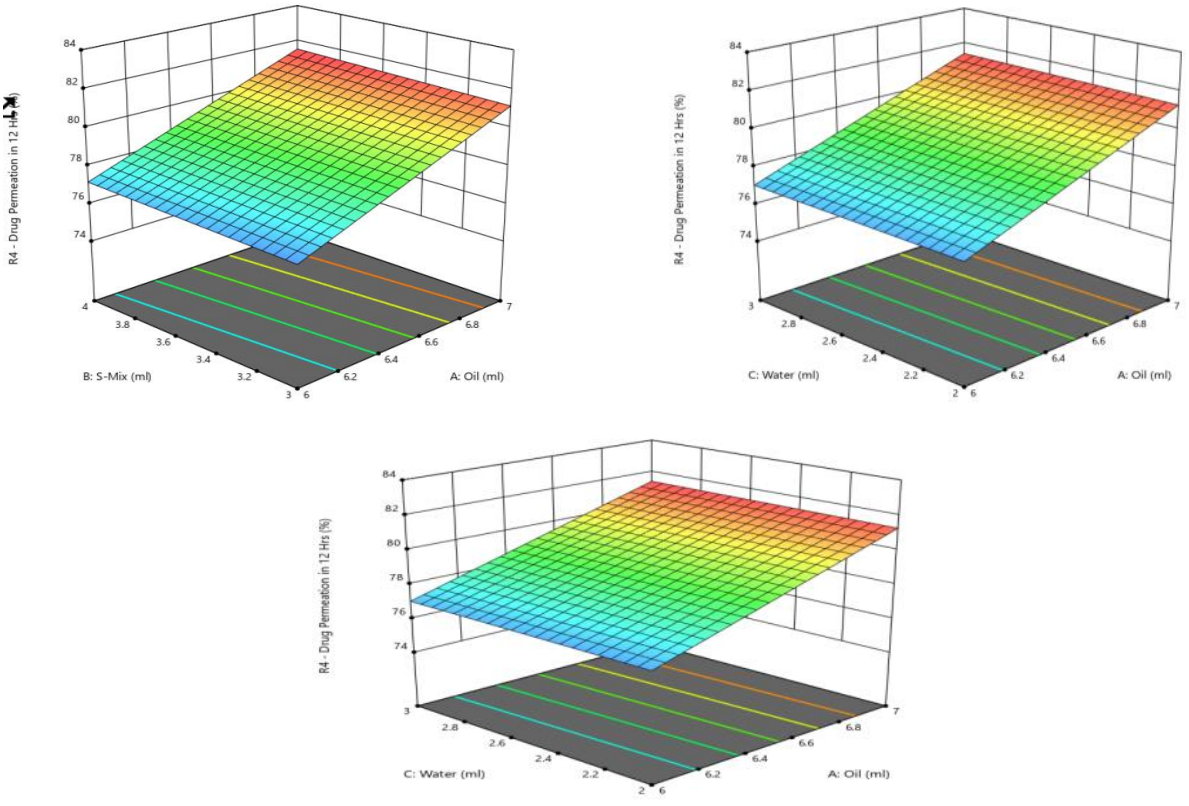


Figure 8: 3D Surface graphs of gaultheria oil- piroxicam microemulsions % CDR for 12 hours verses factors oil, S-mix and water i.e. AB, BC, CA



Evaluation of MEH

Three MEH formulations were prepared from the optimized batch of ME, by keeping the drug and excipients concentration same and varying only the gelling substance HPMC K100 M in different proportions of 1%, 1.5% and 2%. Then they are evaluated for various parameters such as pH, viscosity, spreadability, drug content, *in-vitro* diffusion study, *in-vitro* cumulative drug release for 6 hours (%) and 12 hours (%). The results were depicted as follows

Table 16: Various evaluation parameters of MEH

Parameters	**MEH-A**	**MEH-B**	**MEH-C**
	(n=3)	**(n=3)**	**(n=3)**
pH	7.44 ± 0.03	7.42 ± 0.02	7.43 ± 0.06
Viscosity (cps)	4576 ± 16.21	6847 ± 11.36	8974 ± 14.57
Spreadability (g.cm/sec)	5.16 ± 0.08	4.54 ± 0.01	3.87 ± 0.07
Drug content (%)	94.54 ± 0.14	95.81 ± 0.11	93.39 ± 0.25
In-vitro diffusion (%)	88.47 ± 0.15	93.25 ± 0.09	84.61 ± 0.21
In-vitro cumulative drug release at 6 hours (%)	35.57 ± 0.34	42.21 ± 0.11	34.92 ± 0.58
In-vitro cumulative drug release at 12 hours (%)	85.69 ± 0.43	89.95 ± 0.18	81.47 ± 0.54

All values in mean ± SD

Stability studies of optimized formulation MEH-B after a period of 90 days

The accelerated stability studies were performed for optimized formulation of MEH for 90 days period, as per accelerated stability testing of ICH guidelines & results are as follows.

Table 17: Stability studies of MEH-B for pH and drug content after 90 days.

PARAMETERS		**MEH-B**
		(n=3)
pH	5° C ± 2°C	7.40 ± 0.04
	30° C ± 2° C and 65% RH ± 5% RH	7.41 ± 0.06
	40° C ± 2° C and 75% RH ± 5 % RH	7.40 ± 0.02
Drug content	5°C±2° C	95.67 ± 0.15
	30° C ± 2°C and 65% RH ± 5% RH	95.93 ± 0.10
	40° C ± 2° C and 75% RH ± 5 % RH	95.14 ± 0.25

All values in mean ± SD

Skin irritancy test results

The optimized piroxicam -gaultheria oil MEH (MEH-B) scores obtained for skin irritancy test was found to be 'zero' (0), and results are as follows. Both the commercial cream and MEH-B were comparable, wherein the scores demonstrated 'non-irritant' nature with 'no erythema and no edema'. Thereby proving its suitability for dermal application.

Table 18: Scoring of skin irritancy test results

Formulation	**Time**		
	24 hrs	**48 hrs**	**72 hrs**
Commercial cream	0	0	0
Microemulsion-Based Hydrogel (MEH-B)	0	0	0

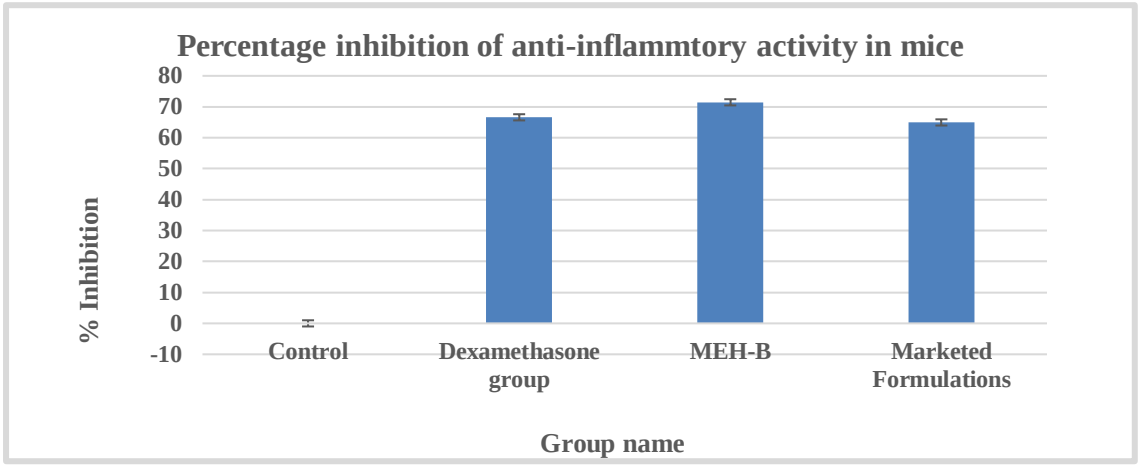
In-vivo anti-inflammatory activity

The anti-inflammatory activity of MEH-B and marketed piroxicam cream were compared and results are as follows.Table 19: Results of *in-vivo* anti-inflammatory activity of MEH-B (by Croton oil induced ear edema model in mice)

Group	**Group name**	**Ear weight (mg) [mean ± SEM]**	**% Inhibition**
I	Control (croton oil 0.5mg/g)	28.10 ± 2.21	-
II	Dexamethasone induced (0.08 mg/ear)	9.34 ± 3.18	66.62 a*
III	MEH-B (0.5% w/w)	8.02 ± 2.16	71.45 b**
IV	Marketed formulation (0.5% w/w)	9.85 ± 3.82	64.95 b*

a * = Dexamethasone induced group in comparison with control group, b * = formulation group in comparison with Dexamethasone induced group, * = statistically significant, ** = statistically very significant.

Figure 9: Graphical representation of *in-vivo* anti-inflammatory activity of MEH-B (by Croton oil induced ear edema model in mice)



***In-vivo* anti-arthritic activity**

The anti-arthritic activity of MEH-B and marketed piroxicam cream were compared and results are as follows.

Table 20: Results of *in-vivo* anti-arthritic activity of MEH-B (by CFA induced paw edema model in rats)

Number	Group name	0 day	4th day	7th day	14th day	21st day
I	Normal control (no drug)	0.11±0.21	0.13±0.82	0.12±0.56	0.12±0.21	0.13±0.08
II	Arthritic control (0.1 ml CFA)	0.18±0.23 a	3.23±0.83 a	3.46±0.42 a	3.89±1.11 a	3.01±0.82 a
III	MEH-B (0.5% w/w)	0.14±0.82 b**	1.84±0.52 b**	1.41±0.18 b**	1.21±0.82 b**	1.01±0.08 b**
IV	Marketed formulation (0.5% w/w)	0.18±0.18 b*	2.56±0.63 b	2.01±0.34 b*	1.81±0.42 b*	1.22±0.31 b*

All values expressed in millimeter (mm) ± SEM;
a * = Arthritic control group in comparison with normal control group, b * = formulation group in comparison with arthritic control group, * = statistically significant, ** = statistically very significant.

Figure 10: Graphical representation of *in-vivo* anti-arthritic activity of MEH-B (by CFA induced paw edema model in rats):

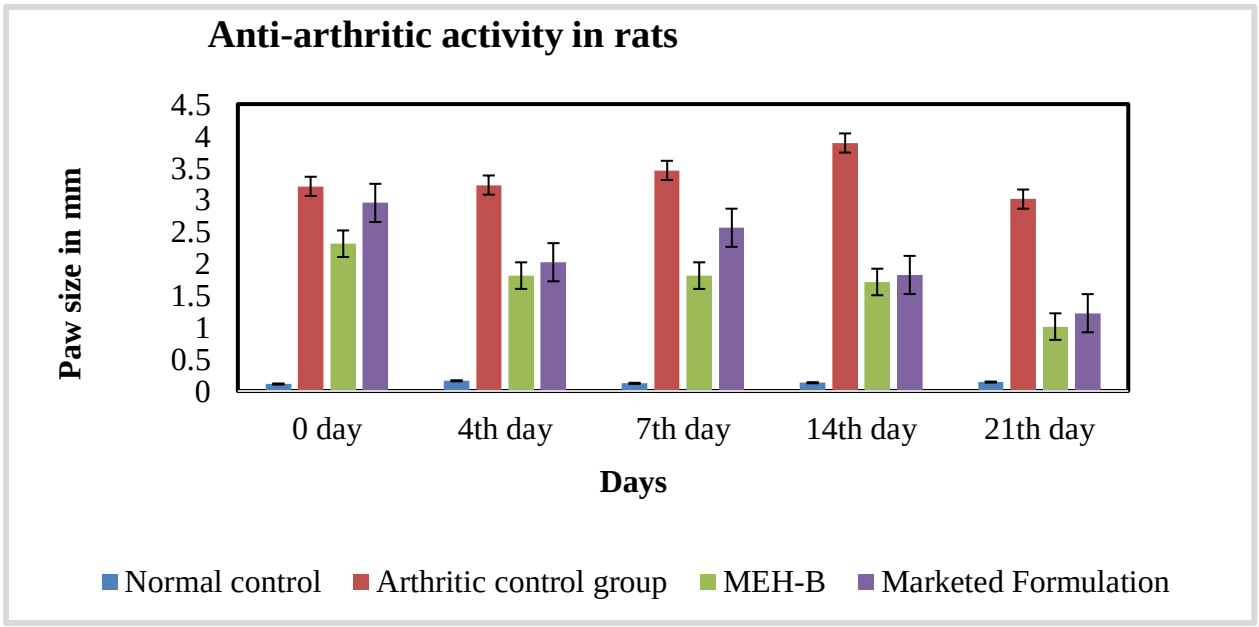


Table 11: Effect of MEH-B on biochemical & hematological parameters in CFA induced arthritis in rats

Parameters	**Normal control**	**Arthritic control**	**MEH-B**	**Marketed formulation**
Hb (g/dl)	16.12 ± 0.43	12.86 ± 0.43 a**	16.01 ± 0.21 b**	14.58 ± 0.43 b*
RBCs (million/µL)	5.23 ± 0.11	4.06 ± 0.03 a**	5.12 ± 0.13 b**	4.72 ± 0.12 b*
Lymphocytes (%)	66.23 ± 0.32	33.56 ± 1.27 a**	65.32 ± 1.34 b**	59.35 ± 1.09 b**
Total Protein (g/dl)	6.52 ± 0.38	4.61 ± 0.11 a*	6.39 ± 0.56 b**	6.23 ± 1.71 b**
ESR (mm after 1 hr)	1.86 ± 0.17	6.51 ± 0.22	2.31 ± 0.31 b**	3.72 ± 1.16 b*
WBC (cells/ µL)	5383 ± 173	8251 ± 321 a**	5401 ± 160 b**	6221 ± 651 b*
Neutrophil (%)	33.60 ± 1.25	65.52 ± 3.56 a**	34.32 ± 1.14 b**	47.51 ± 2.50 b*
SGPT (IU/ L)	90.3 ± 7.61	189 ± 8.13 a**	96.10 ± 6.21 b**	121.32 ± 3.23 b*
SGOT (IU/ L)	130 ± 5.34	201 ± 11.57 a**	132 ± 9.43 b**	156 ± 12.14 b*
ALP (IU/ L)	183 ± 7.62	316.5 ± 29.31 a**	184 ± 5.69 b**	206 ± 10.31 b*
CRP (pg/ml)	10500 ± 20.53	40800 ± 80.36 a**	12300 ± 25.67 b**	15000 ± 32.15 b*

The blood levels of WBCs, neutrophils, and ESR were significantly increased in arthritis-induced rats, which was correlated to earlier studies. This increase in total WBC count in the arthritic group could be due to immune system activation in response to invading pathogenic microorganisms. As a result, inflammatory mononuclear cells have infiltrated the joints of the arthritic control. ESR is an indication of the acute stage response of arthritis. During inflammatory conditions, neutrophils activate and produce mediators, including platelet-activating factor and lysozyme, producing inflammatory disease. ESR can affect a variety of factors, including blood fibrinogen concentration, immunoglobulins, and hemoglobin. A higher ESR level in arthritic rats shows the chronicity and severity of the disease. The MEH-B treatment brought the changes in WBCs, neutrophil count, and ESR level to the normal.

Serum levels of SGPT, SGOT, and ALP were a method for determining the anti-arthritic potential of the test medication. Previous studies indicated the same results and is a characteristic of arthritis. The production of physiologically active chemical mediators such

as bradykinins by SGOT and SGPT is critical in the inflammatory process. The liver and bone fraction isoenzymes were raised in arthritic rats, which resulted in an increase in ALP levels. The MEH-B treatment reduced the rate of increase in serum SGPT, SGOT, and ALP levels.

These findings demonstrate the protective role of MEH-B in reducing bone loss and organ protection in arthritic rats.

SUMMARY

The present research aimed to formulate a novel microemulsion-based hydrogel (MEH) of gaultheria oil for the topical delivery of piroxicam, to enhance solubility, dermal permeability & prolonged release, using novel gaultheria oil, thereby exhibiting increased anti-inflammatory and anti-arthritic activity. The investigation was conducted to address the drawbacks associated with conventional oral drug dosage forms and to introduce a novel hydrogel to the market for the prolonged release of the non-steroidal anti-inflammatory drug (NSAID) Piroxicam. The selection of excipients such as oil, surfactant, and cosurfactant was made based on the solubility studies and pseudo-ternary phase diagram. The formulation and optimization of microemulsion based hydrogel (MEH) was performed by design of experiment (DOE) using D-optimal design expert software and evaluated for qualitative and quantitative tests. The optimized microemulsion (ME) was then formulated into hydrogels using hydroxy propyl methyl cellulose (HPMC) K100M in different proportions and evaluated for pH, viscosity, spreadability, drug content, diffusion study, skin irritancy, *in-vitro* drug release, *in-vivo* anti-inflammatory, and *in-vivo* anti-arthritic activity. The optimized MEH showed a pH of 7.42, viscosity 6847cps, spreadability 4.54g.cm/sec, drug content 95.81%, *in-vitro* diffusion 93.25%, *in-vitro* cumulative drug release (CDR) 42.21% in 6 hours and 89.95% in 12 hours. Finally, *in-vivo* Anti-inflammatory and anti-arthritic activity of the optimized MEH were comparable and found to be more effective than the available marketed gel. Thus, MEH of piroxicam was successfully developed using Gaultheria oil as a novel encapsulating vehicle for prolonged release of the drug.

CONCLUSION

In the present study, microemulsion based hydrogel (MEH) formulations for the dermal delivery of piroxicam was developed using gaultheria oil and characterized. *In-vivo* anti-inflammatory and anti-arthritic activity of MEH-B formulations was significantly higher when compared to conventional marketed Piroxicam cream. This enhanced activity was attributed to the synergistic effect which occurred due to the combined effect of gaultheria oil and NSAID (Piroxicam). In addition, more drug entrapment could be possible by using gaultheria oil as a new encapsulating vehicle. Further, high dermal penetration & prolonged release of the drug for over a period of 12 hours could be achieved using HPMC K100 M. Thus, our study concluded that that MEH formulation of piroxicam could be successfully formulated using gaultheria oil as a novel encapsulating vehicle for enhanced anti-inflammatory and anti-arthritic activity,that could be beneficial in treating arthritis.s

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REVIEW ARTICLE

Perspectives on Transdermal Drug Delivery System: A Review

Vani S[1,2], Venkatesan N[1], Chandrasekar SB[2], Sreedhar C[3], Anil T Pawar[4]

[1]School of Pharmacy, Sri Balaji Vidyapeeth (Deemed to be University),
SBV Campus, Pillaiyarkuppam, Pondicherry - 607402, India
[2]Drugs Testing Laboratory, Drugs Control Department,
Government of Karnataka, Palace Road, Bangalore - 560001, Karnataka, India
[3]Karnataka College of Pharmacy, Hegde Nagar Main Road,
Thirumenahalli Post, Bangalore - 560064, Karnataka, India
[4]School of Health Sciences and Technology,
Dr. Vishwanath Karad MIT World Peace University, Pune - 411038, Maharashtra, India
*Corresponding Author E-mail: **svani2078@gmail.com**

ABSTRACT:

Drug delivery system (DDS) is a collective term used for techniques that carry drugs into or throughout the body for administration and distribution of drug substances to the target cells, specific tissues or desired organs, to exert optimal effects. Among various routes of DDS, the transdermal drug delivery system (TDDS) forms an attractive approach compared to other routes of administration in terms of easy application, non-invasive, avoiding first pass metabolism, for systemic / local effect, directly reaches blood stream, target drug delivery, controlled release of the drug, easy termination of drug action by removing the patch, suitable for geriatric, paediatric subjects, unconscious patients, enhanced subject compliance and decreased side effects and inter and intra-patient variability as well. In this review, we focused on physiology of skin, routes of percutaneous absorption and types of TDDS, design, development and formulation approaches, basic elements, pharmacokinetics, factors affecting drug penetration, characterization and its evaluation. Significant discussion of their specific advantages, benefits especially in pain management, clinical considerations, general guidelines for using TDDS, market demand etc. This article also provides valuable literature for suitability of most of the Non-steroidal anti-inflammatory drugs (NSAIDs) for transdermal administration mainly due to their pharmacological aspects. In recent years, the scope of TDDS has increased in national as well as foreign market. Hence there are increased research activities from many pharma companies and research institutes for potential drug candidates for TDDS. The promising response of transdermal patches has spurred further research and development in this area. The global transdermal patch pipeline is overflowing, pointing to a bright future for these patches in the coming years. Therefore, TDDS may be the best alternative to oral formulations for effective treatment of inflammatory conditions.

KEYWORDS: DDS, TDDS, Skin, Characterization, NSAIDs, pain.

INTRODUCTION:

Drug delivery systems (DDS):

DDS is a collective term used for techniques that carry drugs into or throughout the body for administration and distribution of drug substances to the target cells, specific tissues or desired organs, to exert optimal effects.

In other words, DDS is a device that administers therapeutically active substance or a drug substance into the systemic circulation with enhanced safety and efficacy by controlled release of drugs in the body at a predetermined rate, at a given time and at a specific place[1].

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Original Article

DESIGN, DEVELOPMENT AND EVALUATION OF NOVEL GAULTHERIA OIL MICROEMULSIONS WITH ENHANCED LOADING OF PIROXICAM FOR TOPICAL DELIVERY IN THE TREATMENT OF ARTHRITIS

VANI SETTY[1*], VENKATESAN NATARAJAN[2], SREEDHAR CHANDANAM[3], CHANDRASEKAR SANDANAPALLI BASAVARAJAIAH[4]

[1]Sri Balaji Vidyapeeth, Deemed-to-be University, Pondicherry-607402, India. Drugs Testing Laboratory, Drugs Control Department, Government of Karnataka, India. [2]School of Pharmacy, Sri Balaji Vidyapeeth, Deemed-to-be University, Pondicherry-607402, India. [3]Department of Pharmaceutical Chemistry, Karnataka College of Pharmacy, Bangalore-560064, Karnataka, India. [4]Drugs Testing Laboratory, Drugs Control Department, Government of Karnataka, India

[*]Corresponding author: Vani Setty; [*]Email: vanisramu@gmail.com

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ABSTRACT

Objective: In the present research, the plant oil of *Gaultheria procumbens* (family: Ericaceae), an extensively utilized ethnic drug to treat arthritis and get rid of pain, was selected to formulate Micro Emulsions (MEs). The objective of this research was to examine the suitability of gaultheria oil in formulating MEs containing Non-Steroidal Anti-Inflammatory Drug (NSAID) for topical use.

Methods: A new water-in-oil Piroxicam Micro Emulsions (PMs) consisting of gaultheria oil, tween 20 and Poly Ethylene Glycol (PEG) 600 were formulated using D-optimal design expert software for optimization of formulation and also to enhance the loading capacity of a model NSAID piroxicam has been demonstrated. The pseudo-ternary phase drawing has been depicted on a constant surfactant/co-surfactant (S-mix) ratio (1:1). Then the prepared PMs were evaluated for percentage (%) transmittance, pH determination, refractive index (RI), viscosity measurement, % drug content, particle size, zeta potential, *in vitro* cumulative drug release and stability studies.

Results: The pre-formulation studies showed high solubility of drug (piroxicam) with gaultheria oil, tween 20 and PEG 600, thereby confirming its suitability for formulation. Fourier Transform Infrared Spectroscopy (FTIR) proved drug excipient compatibility by retaining the characteristic peaks even after formulation. D-optimal design expert software was proved to be beneficial in the formulation and optimization process and matched the experimental results. Optimized Piroxicam Micro Emulsion (PM) formulation PM 8 showed satisfactory results regarding % transmittance (98.84±1.04), pH (7.41±0.01), RI (1.4421±0.002), viscosity (469.53±0.61), % drug content (96.32±0.19), particle size (185.5 nm±0.24 nm), zeta potential (-25.34±0.51), *in vitro* cumulative drug release in 6 h (42.62 %±0.11 %) and 12 h (86.42 %±0.21 %). The drug release kinetics study showed a zero-order release mechanism confirmed by Korsmeyer-peppas model. Stability studies as per International Council for Harmonization (ICH) guidelines demonstrated the stability of PM 8 for its physical appearance, phase separation, % transmittance, pH and % drug content for 90 d.

Conclusion: Thus, it can be concluded that the present investigation study can be beneficial for improved loading of model NSAID piroxicam in the interfacial film of PM with gaultheria oil as a promising vehicle aimed at the topical conveyance of drugs in the management of arthritis.

Keywords: Microemulsions, Gaultheria oil, Arthritis, Pseudo-ternary phase drawing, Design expert software

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INTRODUCTION

Arthritis is the most prevailing pain affecting millions of people globally. It develops when the protective cartilage insulating the bones wear out over time [1]. Even though, arthritis can catastrophe any joint, the most affected joints are the spine, hips, knees and hands [2, 3]. The disorders usually progress gradually and deteriorate sometime. Signs and indications of arthritis comprise stiffness, pain and inflammation, grating sensation; loss of elasticity and bone outgrowth leading to swelling. Hence, it should be treated at the earliest to avert bone damage [4, 5].

The genus Gaultheria, consisting of approximately 134 species, is widely engaged as an ethnic drug to treat arthritis and relieve pain. Gaultheria oil is also known as wintergreen essential oil [6]. It is one of the two plants in the world that comprises 'methyl salicylate' (The other plant being *Betula lenta*, family: Betulaceae) [7]. It is the chemical behind the strong soothing properties of gaultheria oil. Phytochemical investigations of previous works on the genus Gaultheria have shown the presence of extensive methyl salicylate derivatives [8]. Methyl salicylate is considered as a characteristic ingredient for its anti-arthritic effects [9]. In this research, MEs for arthritis using gaultheria oil are explored to establish its potential and advance research [10, 11].

Piroxicam is a NSAID belonging to the oxicam group and Bio pharmaceutics Classification System (BCS) Class II drug employed to relieve the indications of tender inflammatory disorders in arthritis. It acts by checking the production of endogenous prostaglandins that mediate swelling, pain, stiffness and tenderness. It is used in the treatment of certain inflammatory conditions like Rheumatoid Arthritis (RA) and Osteo Arthritis (OA) to relieve moderate to severe pain [12-14]. Its effect usually lessens pain and inflammation by performing as a non-selective Cyclo Oxygenase (COX) 1 and 2 Inhibitors. Piroxicam should be taken with caution in patients with liver disease and severe kidney disease since its half-life is 50 h. Hence it is desirable to use transdermal delivery of piroxicam rather than oral administration, for treating arthritis. Presently available forms of piroxicam topical applications have potency of 0.5 % w/v, which is not sufficient for the chronic treatment of arthritis [15-17].

MEs are clear, thermodynamically stable, transparent, optically isotropic systems consisting of oil, surfactant, co-surfactant and an aqueous phase. They work together to form stable MEs, by lowering the interfacial tension. MEs have been stated to advance the rate and magnitude of absorption of lipophilic drugs. Thus, MEs are used as impending drug delivery vehicles due to their reversibility and thermodynamic stability. It has simple manufacturing and scale-up practicability and does not necessitate any distinct equipment. Water-in-oil (w/o) MEs are the most appropriate form that is projected to enhance the solubility of drugs by dissolving into an oil phase [18-21].

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